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OBSERVATIONS ON THE NUTRITION OF SEVEN SPECIES OF RHYNCHOCOELAN WORMS[1]

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Such information as is available concerning nutrition within the phylum Rhynchocoela is largely restricted to reports on the food and feeding mechanisms of relatively few species. The group, which is predominantly free living in habit, is generally regarded as carnivorous or scavenging, and potential food is detected either from a distance by means of chemotactic receptors (Reisinger, 1926; Coe, 1943; Beklemishev, 1955), or at short range by the eyes (Jennings, 1960; Roe, 1967). Active living prey are caught by the proboscis; in the hoplonemerteans stylet bulb secretions frequently cause paralysis or death of the captured organism, but no comparable effect is reported in the palaeonemerteans, heteronemerteans or bdellonemerteans, which lack proboscis armature. Partial proboscis retraction then brings food to the mouth, where it is either swallowed intact or sucked dry of its softer body parts. In contrast, inactive living prey or decaying food materials are ingested directly without prior proboscis eversion (McIntosh, 1873–1874; Du Plessis, 1893; Reisinger, 1926; Coe, 1943; Gontcharoff, 1948; Hylbom, 1957; Tucker, 1959; Jennings, 1960; Hickman, 1963; Roe, 1967).

Digestion has been variously reported as very rapid (Wilson, 1900; Child, 1901; Piéron, 1914; Coe, 1943; Gontcharoff, 1948; Jennings, 1960, 1962a), or as lasting several hours or even up to a few weeks (Du Plessis, 1893; Coe, 1943; Beklemishev, 1955). Reisinger (1926), using histological methods, showed that in the hoplonemertean *Prostoma rubrum* an extracellular proteolytic phase is followed by intracellular proteolysis and lipolysis, with carbohydrases playing only a minor role. Jennings (1962a), using histochemical methods, showed that in the heteronemertean *Lineus ruber* the extracellular phase is acidic and involves cathepsin-C type endopeptidase. This is followed by intracellular completion of digestion by exopeptidases, lipase and carbohydrases.

In contrast to these descriptions of digestion in carnivorous species, Gibson and Jennings (1969) showed that in the entocommensal bdellonemertean *Malacobdella grossa,* an atypical microphagous species with a large proportion of plant material in its diet, the complement of proteolytic enzymes is very much reduced and digestion effected primarily by carbohydrases.

Little is known concerning food reserves in the Rhynchocoela. Only three species have been examined and in all of these fat forms the principal reserve, supplemented by smaller amounts of glycogen (Reisinger, 1926; Jennings, 1960; Gibson and Jennings, 1969).

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It can be seen from this brief review that no comparative account of nutrition within the phylum exists. In the present study, therefore, the diet, feeding mechanisms, digestive physiology and food reserves of seven species representative of the three major orders of rhynchocoelans have been investigated, in order to establish the general pattern of nutrition within the phylum.

Materials and Methods

The following rhynchocoelan species, listed systematically, have been examined:

Anopla

Order palaeonemertini

Cephalothrix bioculata Oersted

Cephalothrix linearis (Rathke)

Order heteronemertini

Lineus ruber (Müller)

Lineus sanguineus (Rathke)

Enopla

Order hoplonemertini

Amphiporus lactifloreus (Johnston)

Tetrastemma melanocephalum (Johnston)

Prostoma rubrum Coe

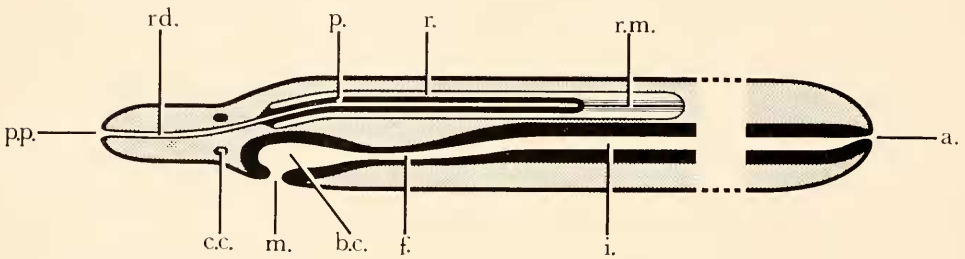


Figure 1. *Cephalothrix bioculata*. Schematic vertical longitudinal section to show the arrangement of the alimentary canal and proboscis, characteristic of the Palaeonemertini. a., anus; b.c., buccal cavity; c.c., cerebral commissure; f., foregut; i., intestine; m., mouth; p., proboscis; p.p., proboscis pore; r., rhynchocoel; rd. rhynchodaeum; r.m., retractor muscle of proboscis.

Prostoma rubrum, the only freshwater species investigated, was obtained from ponds near Stratford, Connecticut through the courtesy of Dr. John J. Polubowich. The other species were collected from the intertidal zones at Filey Brigg and Robin Hood's Bay, on the Yorkshire coast.

For general histological examination specimens were fixed in marine Bouin, Susa, or 10% neutral formalin. Excessive contraction and coiling during fixation was prevented by using these fixatives at 37° C and in some instances prior relaxation in 8% aqueous magnesium chloride was also found to be advantageous. Paraffin sections cut at 6 μ were stained by routine methods, including hematoxylin and eosin, Feulgen, Mallory's trichrome, Mayer's haemalum, Alcian blue (for

mucins), the bromphenol blue method of Mazia, Brewer and Alfert (1953) for proteins, and the periodic acid-Schiff (PAS) method for mucins and carbohydrates.

The nature of the diet was investigated from sections and squashes of the gut of freshly collected specimens, and from preference tests in which each species was presented with representatives of the fauna associated with it in nature. Mechanisms for capturing and ingesting the chosen food were studied by direct observation, particular attention being paid to the condition and type of food that evoked proboscis eversion and to the times required for complete ingestion. Steinmann's fluid was used in many cases to fix specimens in the act of feeding, this fixative acting rapidly enough to prevent proboscis withdrawal or muscular contractions.

The site and sequence of digestion were studied by fixing series of individuals at progressive intervals after an observed meal, breakdown of the food within the gut being followed histologically by the methods listed.

The types of enzymes involved in digestion were investigated histochemically in similar series fixed at 4° C in 10% neutral formalin containing 3% sodium chloride. Sections were cut either directly on a freezing microtome or after rapid dehydration in cold acetone, clearing in xylol and infiltration *in vacuo* in paraffin wax melting point 45° C. Techniques used for enzyme identification included the Hausler (1958) and Hansson (1967) methods for carbonic anhydrase; the Hess and Pearse (1958) method for endopeptidase of the cathepsin-C type as used by Jennings (1962a, 1962b), Rosenbaum and Ditzion (1963) and Jennings and Mettrick (1968); the Burstone and Folk (1956) method for exopeptidases of the leucine aminopeptidase type; the Holt and Withers (1952) and Gomori (1952) methods for non-specific esterases; the Gomori (1952) and Abe, Kramer and Seligman (1964) methods for lipases; the Gomori (1952) and Burstone (1958) methods for acid phosphatase; and the Gomori (1939) method for alkaline phosphatase. Attempts were made to visualize carbohydrase activity using the methods of Pearse (1961) for β-glucuronidase (after Fishman and Baker, and Seligman, Tsou, Rutenburg and Cohen), and for α-glucosidase (after Rutenburg, Lang, Goldburg and Rutenburg).

Controls for these histochemical methods included the use of heat inactivated sections, media lacking specific substrates or containing specific activators or inhibitors, and the simultaneous processing of appropriate mammalian tissues.

The distribution of carbohydrate reserves was investigated in specimens fixed in 90% alcohol containing 1% picric acid, sections being stained by the Best's carmine or PAS methods for glycogen. Fat reserves were studied after fixation in Flemming's fluid or in frozen sections of formalin-fixed material stained in Oil Red O or Sudan Black B.

Observations

Anopla

Order: palaeonemertini

Cephalothrix bioculata and *C. linearis*

The two palaeonemertines studied show no significant differences in the structures and physiological processes concerned with nutrition and the following account is applicable to both species, unless stated otherwise at the appropriate point.

Structure of the gut and proboscis

The alimentary canal in both species is ciliated throughout its length and divisible morphologically into three distinct regions, the mouth and buccal cavity, the foregut, and the intestine (Fig. 1). The mouth is ventral and subterminal, 2–4 mm from the anterior end, and elliptical with its long axis running transversely to the main body axis. The deeply lobed lips and some slight folding of the buccal epithelium anteriorly allow the mouth to be distended during ingestion to a diameter equal to, or even slightly in excess of, the diameter of the body.

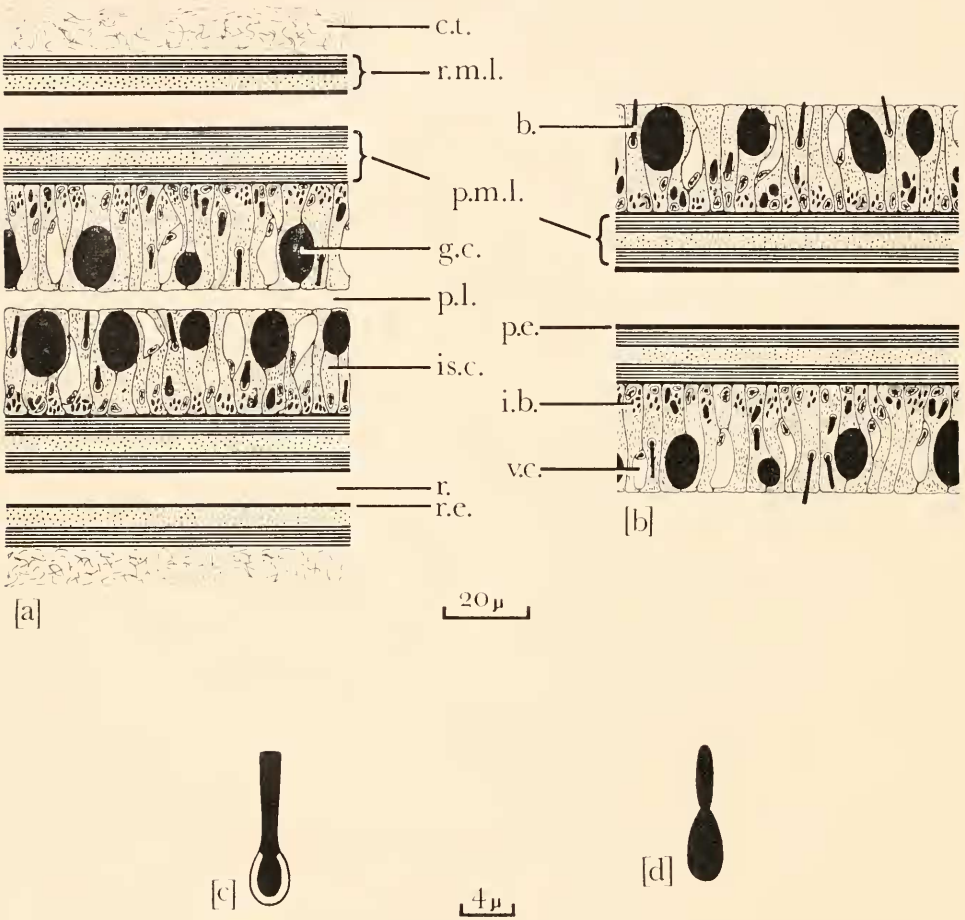


FIGURE 2(a). *Cephalothrix bioculata.* Schematic vertical longitudinal section through a portion of the proboscis and rhynchocoel as seen in the retracted position. (b) *Cephalothrix bioculata.* Schematic vertical longitudinal section through a part of the proboscis in the everted condition. b., barb; c.t., connective tissue; g.c., granular cell; i.b., immature barbs; is.c., interstitial cell; p.e., proboscis endothelium; p.l., proboscis lumen; p.m.l., proboscis muscular layers; r., rhynchocoel; r.e., rhynchocoel endothelium; r.m.l., rhynchocoel muscular layers; v.c., vacuolate cell. (c). Proboscis barb from *Cephalothrix bioculata.* (d). Proboscis barb from *Cephalothrix linearis.*

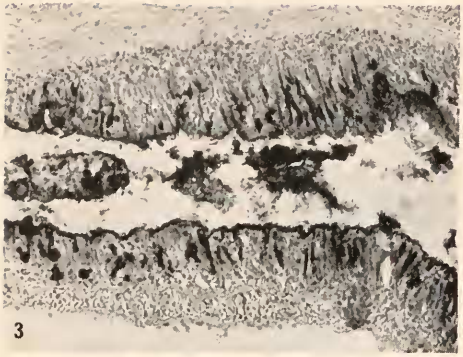
The buccal epithelium is made up of ciliated columnar cells 50–60 μ tall and 4–6 μ wide, with acidophilic and basophilic gland cells 18–20 μ by 5–7 μ interspersed amongst them. The majority of the basophils produce mucoid secretions, staining strongly with Alcian blue and PAS, which are discharged during ingestion presumably to facilitate passage of the food. The acidophils, in contrast, produce non-mucoid secretions and are extremely rich in carbonic anhydrase (Fig. 3), an enzyme normally associated in alimentary systems with production of hydrochloric acid. Cytoplasmic carbonic anhydrase can be demonstrated at all times, and when the acidophils discharge during ingestion of food the enzyme can also be found in the secretions poured on to the food as it passes onwards into the foregut.

The foregut occupies about one-fifteenth of the animal's length and consists of a simple unfolded ciliated tube. The epithelium is identical with that of the buccal cavity as regards both nature and frequency of the cell types present, but is reduced in height to 25–30 μ. The lumen is approximately 30 μ in diameter, narrowing to 15–18 μ at the junction with the intestine.

The intestine is the longest part of the gut and extends from its junction with the foregut direct to the anus at the posterior extremity. There is no cecum, but paired shallow lateral diverticula occur for most of the length up to a short pre-anal region which lacks these structures. The intestinal wall, or gastrodermis (Fig. 4), is composed of two cell types arranged as a single layer upon a thin basement membrane. The majority of the cells are columnar, 50–60 μ tall and 5–7 μ wide, sparsely ciliated and with prominent basal nuclei. Elongate pyriform gland cells, 35–40 μ by 7–8 μ and packed with acidophilic proteinaceous spheres 1.5 μ or less in diameter, occur proximally between the columnar cells. The spheres show a strong positive reaction to the Hess and Pearse method for endopeptidase of the cathepsin-C type (Fig. 4) and to the Holt and Withers, and Gomori, methods for non-specific esterase. Tracts of spheres often appear between the columnar cells, extending distally from the gland cells to the gut lumen. Smaller, more club-shaped forms of these gland cells also occur in the gastrodermis but in these the contained spheres are basophilic and react only weakly with the methods for enzyme visualization. Such cells never show tracts of discharged spheres leading from them towards the gut lumen and it would appear that they represent younger gland cells in the process of maturation.

Mature gland cells are abundant throughout most of the intestine but decrease in number posteriorly. Thus in the anterior region of the intestine in *C. bioculata*, for example, between 160 and 180 occur per 100 μ^2 of the gastrodermal surface, with a ratio of gland cells to columnar cells of about 1:4. Towards the anus, however, these values decrease to 30–35 and 1:20, respectively. In *C. linearis* the gland cells tend to be even more concentrated towards the anterior end of the intestine and here the ratio with the columnar cells is about 1:3, decreasing to 1:60 posteriorly.

The proboscis (Fig. 1) in both species in unarmed. It is a musculo-glandular tubular structure, which when retracted lies within the proboscis chamber or rhynchocoel and is confluent with the rhynchodaeum. This is a narrow tube opening to the exterior at the terminal proboscis pore. The proboscis can be everted through the rhynchodaeum by increased pressure within the fluid-filled rhynchocoel, and subsequently retracted by release of this pressure and contraction of the retractor muscle.



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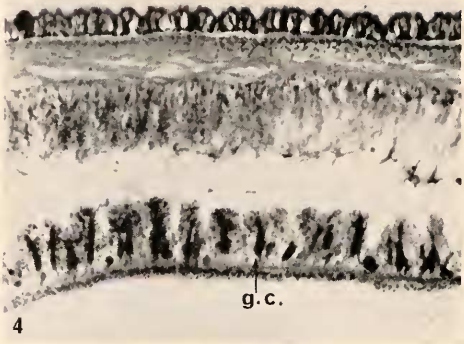

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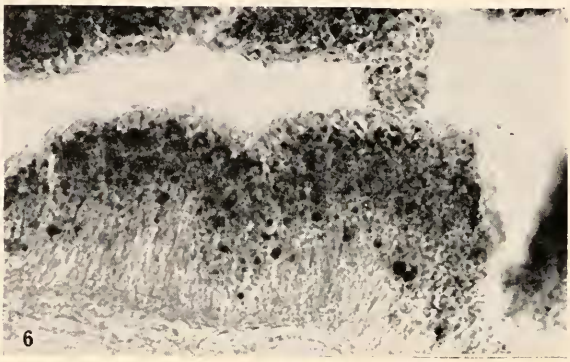

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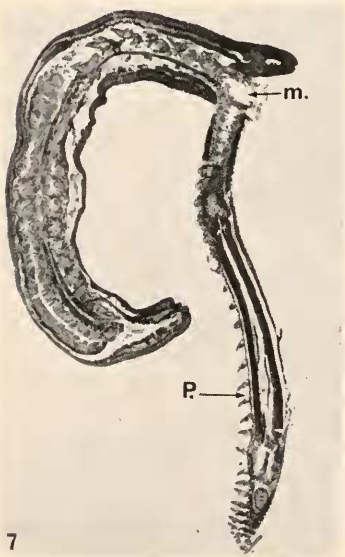

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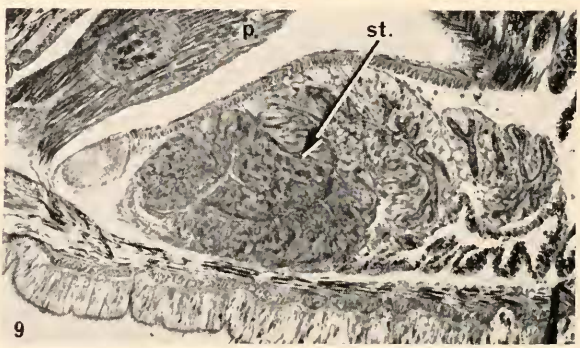

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FIGURES 3–9.

The rhynchocoel is lined by a thin endothelium overlying a thin band of muscle which in *C. bioculata* is composed of an inner layer of circular and an outer layer of longitudinal fibers. In *C. linearis* the outer muscular layer is oblique rather than longitudinal. The proboscis itself, in the retracted condition, is enclosed in a similar endothelium but possesses three muscular layers consisting of inner circular fibers surrounded on either side by longitudinal ones (Fig. 2a, b). The proboscis epithelium forms a smooth covering to the proboscis and is not thrown into papillae such as occur in the enoplan rhynchocoelans. In *C. bioculata* it is composed of three cell types, and four in *C. linearis*. In both species the commonest cell type is the interstitial cell, which is columnar, 25–30 μ tall and 3–8 μ in width. The distal cytoplasm contains up to ten acidophilic, proteinaceous and PAS positive rod-like structures, or barbs, very reminiscent of turbellarian rhabdites. When fully developed the barbs in *C. bioculata* (Fig. 2c) are 12 μ long and 1–1.5 μ wide with a bulbous proximal end which is inserted into a refractile cup 4–5 μ long and 2.5–3 μ wide. The point of insertion of the barb into the cup is in many instances distended into a slight collar-like structure. The barbs are arranged distally in tetrads and when the proboscis is everted they are protruded from the interstitial cells (Fig. 2b).

The barbs in *C. linearis* (Fig. 2d) are only 9–10 μ in length and lack a basal cup, but show a marked waist or constriction just over halfway along their length. They are not grouped into tetrads, but are occasionally paired.

The interstitial cells also contain proximally 8–10 simple rods which are considerably smaller than the barbs of the distal region but show the same staining reactions. They are believed to be the precursors of the fully differentiated barbs and presumably migrate distally to replace those protruded during proboscis eversion.

The other cell types present in the proboscis epithelium are oval vacuolated cells, 10–12 μ wide, and spherical forms which lack a nucleus when mature and

FIGURE 3. *Cephalothrix linearis*. Longitudinal section through a portion of the buccal cavity (right) and foregut to show the acidophilic glands (black) which are rich in carbonic anhydrase. Hausler's method. Scale: 1 cm = 25 μ.

FIGURE 4. *Cephalothrix bioculata*. Longitudinal section of the gastrodermis. g.c., a gland cell showing a strong positive reaction for endopeptidase, lying between columnar cells. Hess and Pearse method. Scale: 1 cm = 50 μ.

FIGURE 5. *Cephalothrix linearis* photographed ingesting a newly captured *Tubifex*. The head (h.) is held upwards away from the mouth, which is distended into a funnel-shape and is grasping the *Tubifex* near its posterior end. Scale: 1 cm = 4 mm.

FIGURE 6. *Cephalothrix bioculata*. Longitudinal section of the gastrodermis prepared four hours after feeding and showing acid phosphatase activity (black) in the food vacuoles lying in the distal half of the columnar cells. The cytoplasm around the vacuoles shows a similar but less intense reaction. Burstone's azo dye method. Scale: 1 cm = 20 μ.

FIGURE 7. *Lineus sanguineus*. Longitudinal section of an individual fixed in Steinmann's fluid three hours after commencing ingestion of a *Phyllodoce* (P.). The nemertean's intestine is filled almost to the anus and the portion of the *Phyllodoce* still uningested is being separated off at the mouth (m.). Mallory. Scale: 1 cm = 0.5 mm.

FIGURE 8. *Lineus ruber*. Longitudinal section through the body wall, showing a narrow band (black) of non-specific esterase activity in the extreme distal region of the epidermis. Gomori's α-naphthyl acetate method. Scale: 1 cm = 50 μ.

FIGURE 9. *Amphiporus lactifloreus*. Longitudinal section through the anterior end showing a portion of the proboscis (p.) and the much-folded stomach (st.). Mallory. Scale: 1 cm = 50 μ.

possess very granular cytoplasm. These two cells form the supporting structure of the proboscis and are generally partially covered distally by the interstitial cells. *C. linearis* possesses a fourth cell type, irregular in shape, 15–20 μ in diameter and with acidophilic contents which are often aggregated into ovoid structures similar to the bases of the barbs. No indication of the function of these structures could be obtained.

The lumen within the retracted proboscis, lined by the epithelial layers, contains a coarsely granular lightly PAS positive secretion, and this is extruded when the proboscis is everted and forms a layer covering the epithelium and its protruding barbs. The source of this secretion is unknown, but it may originate from those cells of the proboscis epithelium which are not concerned in formation of the barbs.

The food and feeding mechanism

In the laboratory both species fed readily on living or dead oligochaetes. The freshwater *Tubifex* was convenient for use in observations on the feeding mechanism and was readily taken, but littoral forms such as *Clitellio arenarius* and littoral nematodes (*Pontonema sp.*) were also eaten. Freshly collected specimens generally showed little recognizable material amongst their gut contents but on occasion spherical structures, 1.5–2 μ in diameter and packed with small black granules, were found. These were very similar in size and staining properties to the chloragogenous cells present in most oligochaetes, including *C. arenarius*, and their presence in the intestine is taken as an indication that both species, under natural conditions, feed on littoral oligochaetes and similar organisms.

Both species lack cephalic furrows, which when present in other rhynchocoelans are believed to be the sites of chemoreception (Hyman, 1951), but despite this the feeding behavior indicates that prey is located chemotactically rather than as a response to mechanical disturbance of the water. Dead or damaged organisms are located and seized more quickly than living ones, but the introduction of oligochaetes in any condition elicits the same type of response. Individuals previously quiescent or merely moving slowly around their container become extremely restless when *Clitellio* or *Tubifex* are added, the head darts to and fro and often the body contracts and expands violently. The increased rate of movement soon brings the rhynchocoelan into contact with the food, but they do not "home" on to it as, for example, do many of the free-living flatworms. Introduction of inert, odorless objects does not elicit feeding behavior, and violently threshing prey are definitely avoided. Thus it would appear that food is detected chemotactically but the response is fairly generalized and depends entirely upon an increased rate of random movement.

When within range of living prey the proboscis is everted with explosive force and coils tightly around the prey's body. The prey is then drawn back towards the mouth by retraction of the proboscis, a process which may occupy up to thirty seconds, and during this time the prey becomes inert and apparently lifeless. Examination of the everted proboscis shows that in both species the barbs of the proboscis epithelium are protruded and penetrate the prey's integument. The barbs alone may be responsible for paralyzing the captured animal, but since a considerable quantity of secretions are always present in the lumen of the retracted

proboscis it seems likely that these too are involved, and it is possible that the function of the barbs in this connection may be solely to puncture the prey and allow entry of proboscis secretions. An important secondary function may be to increase the grip of the proboscis on the struggling prey.

Inert foods, such as dead oligochaetes or other animal remains, do not cause proboscis eversion. Such materials, and killed prey brought to the mouth by the proboscis, are first "tested," the head being arched over them and moved slowly from side to side. The head is then bent back, the distended mouth is applied to the food and ingestion commences (Fig. 5). Ingestion is by suction, resulting from alternate contractions and expansions of the general body musculature, and is facilitated by copious secretions of mucus from the basophilic glands of the buccal cavity and foregut. Oligochaetes up to two-thirds the length of the nemertean were completely swallowed within three minutes and could be seen extending into the posterior intestine after a further three or four minutes.

Diameter, rather than length, of the food relative to the size of the mouth appears to be the critical factor determining whether or not ingestion is possible. Thus oligochaetes considerably longer than the nemertean will be swallowed, if

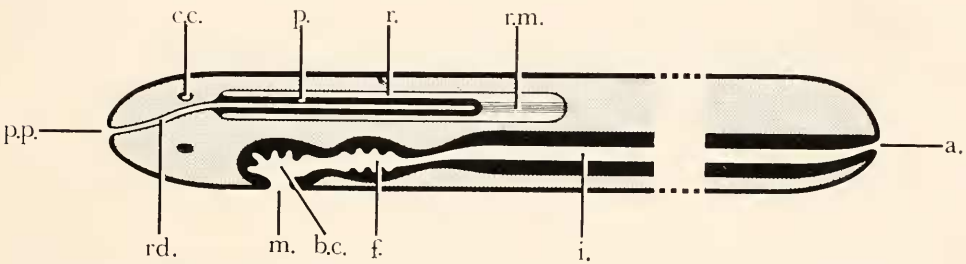


FIGURE 10. *Lineus sanguineus.* Schematic vertical longitudinal section to show the arrangement of the alimentary canal and proboscis, characteristic of the Heteronemertini. Abbreviations as in Figure 1.

of a suitable diameter, and when the foregut and intestine are completely filled the portion remaining protruding through the mouth is nipped off. This takes a little time and may well be caused by regurgitated digestive juices, supplemented by the constricting effect of the contracting mouth.

The site and sequence of digestion

The acidophilic glands of the buccal cavity and foregut, which are rich in carbonic anhydrase (Fig. 3), discharge during ingestion. Foods stained with indicators show that the pH is considerably reduced as material passes on into the intestine and since carbonic anhydrase is known to be associated with acid production in most alimentary systems it is concluded that this is its role in the two species of *Cephalothrix* studied, the acid produced presumably facilitating subsequent proteolysis.

The intestinal acidophilic gland cells, whose contents react strongly with the methods for endopeptidase and non-specific esterase, discharge as food enters the

intestine and their secretions retain their spherical form for a time. Within twenty minutes of feeding, however, the spheres have dissolved and previously enzymically inert boiled food begins to show peripheral endopeptidase activity as proteolysis commences. Eventually the entire contents of the intestine show this reaction, and become progressively more homogeneous, but no other enzymes could be demonstrated. Thus it would appear that extracellular digestion is entirely proteolytic and during this stage the pH of the gut contents is 5.5–6.0, as determined by application of indicators to samples withdrawn by means of a micropipette.

The duration of extracellular proteolysis depends directly upon the size of the meal. Phagocytosis of food particles commences within thirty minutes of feeding, as soon as proteolysis in the gut lumen begins to break up the food, and this continues until the columnar cells of the gastrodermis are loaded with food vacuoles. Material showing endopeptidase activity may still persist in the gut lumen at this stage if a large amount of food has been taken, but as intracellular digestion in the earlier food vacuoles is completed so new ones form distally in the columnar

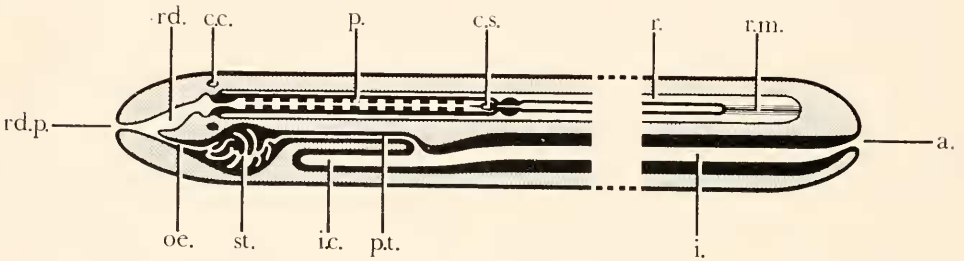


FIGURE 11. *Amphiporus lactifloreus*. Schematic vertical longitudinal section to show the arrangement of the alimentary canal and proboscis, characteristic of this type of Hoplonemertini. c.s., central stylet; i.c., intestinal cecum; oe. esophagus; p.t., pyloric tube; rd.p., rhynchodaeal pore; st., stomach. Other abbreviations as in Figure 1.

cells and this process continues until the lumen is emptied. This generally occurs within twelve hours of feeding but well before this time the lumen contents become quite homogeneous and nothing recognizable remains.

Contents of the food vacuoles continue to show endopeptidase activity for up to four hours after their formation but there is no evidence for the intracellular secretion of the enzyme or enzymes responsible. It is concluded, therefore, that the activity visualized in the vacuoles results from the enzymes originally acting extracellularly and phagocytosed with the digesting food. The vacuoles do, however, develop a strong reaction for acid phosphatase and a less intense but still easily visible reaction is found in the surrounding cytoplasm (Fig. 6). The role of this enzyme remains unknown, but it may be concerned with maintenance of the requisite acidic pH conditions in the vacuole, or perhaps with absorption of some early products of intracellular digestion.

Three to four hours after commencement of phagocytosis exopeptidases, as demonstrated by the Burstone and Folk method for aminopeptidases of the leucine aminopeptidase type, appear in the food vacuoles and rapidly increase in amount

until after a further three hours, when virtually every vacuole in the gastrodermis shows an intense positive reaction. During this time vacuolar reactions for endopeptidase, non-specific esterase and acid phosphatase gradually decline and finally disappear. Eventually the aminopeptidase reaction similarly declines, as intracellular digestion progresses and the food vacuoles become reduced in size and number, and when digestion is completed and all vacuoles have disappeared exopeptidases cannot be visualized in any part of the gastrodermis.

Unlike the exopeptidase reaction, which can only be found in the gastrodermis during the later stages of intracellular digestion, a strong reaction for alkaline phosphatase can be obtained at all times in the distal regions of the columnar cells. Even in long-starved specimens the gastrodermis shows a clearly defined zone of activity distally, 2–4 μ in depth. During the formation of food vacuoles, however, this band of activity deepens and intensifies, and at the peak of exopeptidase activity the enzyme can be demonstrated in the cytoplasm throughout the cell and in virtually every food vacuole. On completion of intracellular digestion the activity fades and becomes confined once more to a thin distal band.

The distribution of alkaline phosphatase in the columnar cells, dependent as it is upon the particular stage of digestion of any one meal, suggests that this enzyme has two main functions. The first of these is concerned with normal cellular activities in the distal region of the cells, such as maintenance and renewal of the cilia, while the second is concerned either with production of the alkaline conditions necessary for exopeptidase activity or with secretion of these enzymes and the subsequent absorption of the products of digestion from the vacuoles.

Lipases and carbohydrases could not be demonstrated in the food vacuoles, but in the early stages of intracellular digestion mucus can be demonstrated in the distal regions of the columnar cells. This presumably represents a proportion of the mucus secreted by the buccal and foregut basophils, to facilitate ingestion, which has been phagocytosed along with food particles. The carbohydrate component of the mucus causes it to stain very strongly with both Alcian blue and PAS, but in later, older, vacuoles these reactions diminish very sharply and then disappear. Thus the presence of carbohydrases can be inferred, at least, in the absence of more direct evidence.

Food reserves

Fat forms the only significant food reserve in both *C. bioculata* and *C. lincaris*. It occurs as droplets, varying in diameter from 1 to 5 μ, in the gastrodermal columnar cells and to a lesser extent in the parenchyma. The amount present at these sites decreases with starvation.

Very small amounts of glycogen were found in the gastrodermis of both species, occurring as minute particles scattered throughout the columnar cells.

Other sites of enzymic activity

In addition to the sites of enzymic activity in the alimentary system certain other sites within the body showed positive reactions to some of the enzyme visualization techniques.

In particular, the endothelial and gelatinous layers of the blood vascular system showed consistently in all specimens examined an intense positive reaction to the Burstone and Folk method for exopeptidases of the leucine aminopeptidase type. The reaction occurs completely independently of the nutritive state of the nemerteans, and of other factors such as the age or reproductive state. Full details of this phenomenon, which is common to all the rhynchocoelans examined in the present study, have been reported in a separate account (Gibson and Jennings, 1967).

Alkaline phosphatase activity was found in the parenchyma immediately adjoining the intestinal basement membrane, where it may well be concerned with transfer of nutrients from the columnar cells into the parenchyma, and in the endothelium and musculature of the rhynchocoel and proboscis. As in the case of the exopeptidases of the blood vascular system, this enzymic activity also is independent of the nutritive state or other discernable factors.

A weak reaction for non-specific esterase occurs at all times in the distal regions of the epidermis in both species, and slightly stronger reactions occur in the proboscis musculature and, occasionally, in the interstitial cells.

Order: HETERONEMERTINI

Lineus sanguineus and *L. ruber*

Structure of the gut and proboscis

The structure of the gut in *L. ruber* has been described in an earlier communication (Jennings, 1960) and consequently details need not be included here. *L. sanguineus* shows virtually no significant differences from *L. ruber*. Briefly, the gut in both species resembles that of *C. bioculata* and *C. linearis* in being divided into buccal cavity, foregut and intestine, but the walls of the buccal cavity and foregut are thicker and thrown up into prominent folds (Fig. 10). A further point of difference lies in the gland cells associated with the buccal cavity and the foregut, in that in the lineid species a considerable proportion of these occur in the underlying parenchyma and discharge through the gut wall into the lumen. These parenchymal gland cells include both basophilic and acidophilic types, the basophils producing mucoid secretions and staining strongly with Alcian blue and PAS. The acidophils are negative to Alcian Blue, stain only lightly with PAS, and their function remains unknown. Of the gland cells contained in the wall of the buccal cavity and foregut, the basophils similarly produce mucus and the acidophils are believed to produce acidic secretions used in killing the ingested prey, since they are rich in carbonic anhydrase.

The intestine, as in the two species of *Cephalothrix*, forms the longest part of the gut and, apart from short anterior and posterior portions, bears serially repeated lateral diverticula. These are up to 700 μ deep, compared with an intestinal width of only 600–650 μ and are frequently bifid distally. The anterior 10–12 pairs, and the posterior 10–16, are shallower and never bifurcated. In an average sized sexually mature adult *L. ruber* the diverticula give a three-fold increase to the internal surface area of the intestine, if the latter is considered as a central cylinder from which lateral pairs of smaller, closed cylinders emerge.

The intestinal wall, or gastrodermis, is identical in both the main region and the diverticula. As in the two *Cephalothrix* species, it is composed of ciliated columnar cells, 80–100 μ tall and 6–8 μ wide in *L. ruber* and 55–70 μ by 4–6 μ in *L. sanguineus,* and smaller pyriform acidophilic gland cells which lie proximally between the columnar cells. The gland cells contain spherical or oval acidophilic, proteinaceous globules showing strong positive reactions to the methods for endopeptidase and non-specific esterase. Tracts of secreted globules extend from the gland cells between the columnar cells up to the intestinal lumen. The gland cells are most numerous in the anterior intestine and decrease in frequency posteriorly, being completely absent from the short unpouched region immediately before the anus.

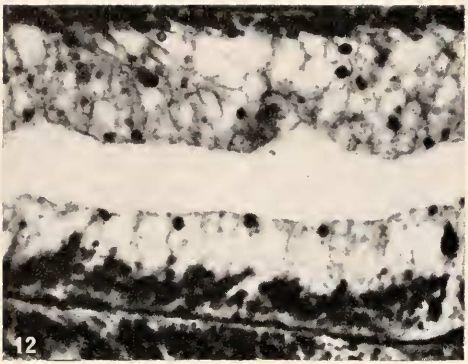
The proboscis (Fig. 10) in both species is unarmed and longer than in either *C. bioculata* or *C. linearis.* It lies coiled within the rhynchocoel, which runs dorsally above the intestine for most of the body length, and the principal difference between it and the cephalothricid type lies in the form of the epithelial rods. These are formed proximally in basophilic interstitial cells as tiny acidophilic rods, undifferentiated morphologically, which migrate to the distal border of the cells where they accumulate in batteries of fifty or more. They may be partially extruded in the retracted proboscis and in the fully everted one they are completely ejected from the formative cells. Their function would appear to be simply to increase the grip of the proboscis on the prey and not to facilitate entry into the latter of toxic secretions. Neither *L. sanguineus* nor *L. ruber* paralyze or kill the prey during capture, and the proboscis secretions other than the acidophilic rods serve merely to increase the effectiveness of the proboscis in gripping and holding a captured organism.

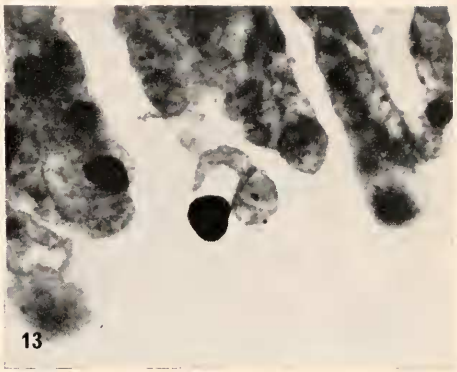
The food and feeding mechanism

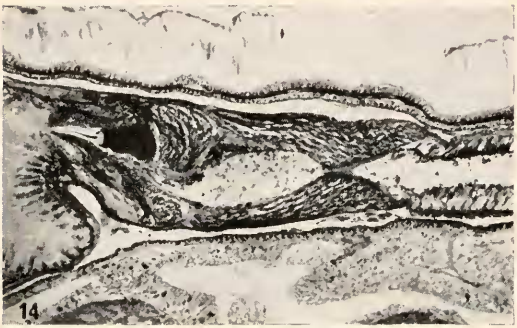
The two species of *Lineus* studied show interesting differences in food preferences. *L. ruber* feeds readily on living or dead oligochaetes, polychaetes, small crustaceans or any attractive organic material such as animal remains or clotted blood. In contrast, *L. sanguineus* will take only living food and feeds most readily on polychaetes such as *Phyllodoce maculata* and various syllids. Oligochaetes such as *Tubifex* or *Clitellio* are taken when the animal is starved, and occasionally other species of rhynchocoelans (*e.g., Amphiporus lactifloreus*) are also taken, but these are never captured and ingested as readily as polychaetes.

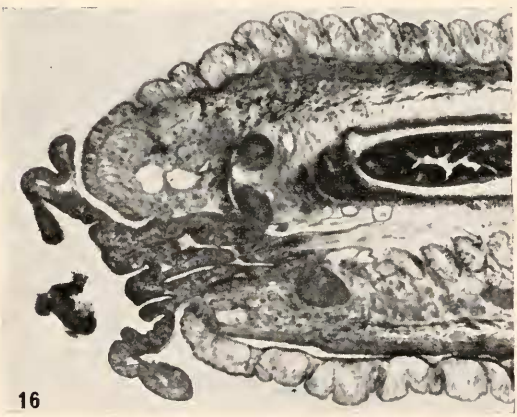
Both species detect prey chemotactically, and are able to locate damaged animals at distances of up to 8 cm. Intact living prey are detected visually within 2–3 cm of the head.

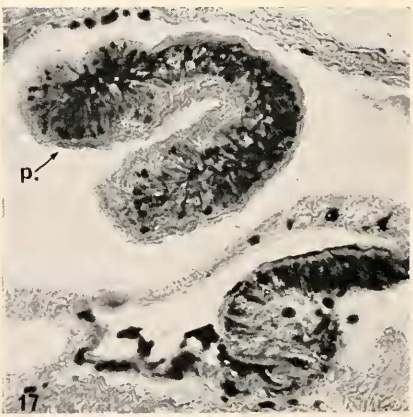
In both *L. sanguineus* and *L. ruber* living animals are captured by the proboscis. This is ejected when the prey is within range, coiled tightly around it and then retracted slowly to draw the struggling animal back to the head. The mouth is subterminal and the portion of the head anterior to it arches upwards and forwards to expose and dilate the mouth. The arched portion of the head may be used to grip the prey and hold it to the mouth, or it may be held clear. Small prey are swallowed within one minute of capture, head or tail first or in a U or J shape depending on the part of the body seized by the proboscis. Ingestion of larger organisms takes longer and *L. sanguineus* on one occasion took three hours to











FIGURES 12–17.

FIGURE 12. *Amphiporus lactifloreus.* Longitudinal section of the gastrodermis in the posterior intestine of a starved specimen, showing columnar cells with basal acidophilic spheres which are the sites of endopeptidase activity. Mallory. Scale: 1 cm = 50 μ.

FIGURE 13. *Amphiporus lactifloreus.* Distal region of the gastrodermis of a specimen fixed 15 minutes after the start of a meal, showing the discharge of an endopeptidase-positive sphere into the gut lumen. Hess and Pearse method. Scale: 1 cm = 10 μ.

ingest part of a *Phyllodoce* which was considerably longer than itself. Ingestion continued until the entire intestine was filled (Fig. 7). This particular specimen was fixed and sectioned at this point, but in other similar instances the portion of the prey left protruding from the mouth was eventually nipped off and freed. Severance of the uningested portion appears to be effected partly by constriction of the mouth and partly by solution of the prey's body by regurgitated digestive juices, judging from the appearance of the end region of the discarded food.

The prey is ingested alive, thus confirming the absence of toxic proboscis secretions, but death usually occurs either as it passes through the foregut or very soon after entry into the intestine.

L. sanguineus refused all inert foods, but these were readily taken by *L. ruber*. As with *C. bioculata* and *C. linearis,* though, the proboscis was never everted and the nemertean simply extended the pre-oral portion of the head, dilated the mouth and engulfed the food.

The site and sequence of digestion

Animals ingested alive generally die as they pass through the foregut and experiments with indicators show that at this time the pH drops to around 5.0. Sections of newly fed individuals show that the acidophilic glands of the buccal cavity and foregut, which are rich in carbonic anhydrase, have discharged and it is concluded that the acidic secretions responsible for the drop in pH emanate from these cells. The mucus-producing basophils also discharge and considerable amounts of mucus can be found around the ingested food.

The endopeptidase-producing acidophils of the gastrodermis discharge as the food enters the intestine and previously enzymically inert materials soon show a positive endopeptidase reaction. From this point breakdown of the food follows virtually the same course as in the two *Cephalothrix* species, extracellular endopeptidase digestion being followed by phagocytosis and completion of proteolysis within the food vacuoles by exopeptidases demonstrable by the Burstone and Folk technique. The sequence of intracellular digestion, with the demonstration of exopeptidases, carbohydrases and lipases acting in concert, has been described in detail for *L. ruber* elsewhere (Jennings, 1962a) and *L. sanguineus* shows no significant differences. This earlier account, however, fails to report the occurrence of acid phosphatase in the gastrodermis and in the present work, using the

FIGURE 14. *Amphiporus lactifloreus.* Longitudinal section through the central region of the retracted proboscis, showing a portion of the anterior proboscis (left), the median bulbous region which contains the central stylet and its base and is expanded into a muscular bulb posteriorly, and a portion of the posterior proboscis (right). Mallory. Scale: 1 cm = 300 μ.

FIGURE 15. *Amphiporus lactifloreus.* Specimen photographed while feeding on a *Gammarus.* The head has been inserted within the crustacean, the stomach everted and contractions of the rhynchocoelan's general body musculature have begun to withdraw the prey's body contents. Scale: 1 cm = 2 mm.

FIGURE 16. *Amphiporus lactifloreus.* Longitudinal section through the anterior end of an individual fixed in Steinmann's fluid while feeding. The stomach is partially everted through the rhynchodaeal pore. Mallory. Scale: 1 cm = 100 μ.

FIGURE 17. *Prostoma rubrum.* Longitudinal section through the stomach (bottom right) and a part of the proboscis (p.) showing localization of carbonic anhydrase activity (black). Hausler's method. Scale: 1 cm = 75 μ.

Burstone azo-dye technique, this enzyme was found to be present in the cytoplasm of the columnar cells even during starvation. On formation of the food vacuoles the cytoplasmic activity shows a marked increase and large amounts of acid phosphatase appear in the food vacuoles. As with the two species of *Cephalothrix,* no definite role could be ascribed to this enzyme other than the suggestion that it may be concerned with maintenance of a low vacuolar pH or the absorption from the vacuoles of some earlier products of intracellular digestion.

Alkaline phosphatase activity occurs in the gastrodermis of both species, independently of the nutritive state, as a thin distal band. This increases in depth as the food vacuoles form and gradually increases in intensity in vacuoles and cytoplasm to a peak which coincides with the peak of exopeptidase activity. Thus in the early vacuoles both acid and alkaline phosphatases can be demonstrated but the relative strengths vary with time, the acid enzyme declining as the alkaline one increases.

Digestion is completed twelve to twenty-four hours after feeding, depending upon the amount of food taken, and indigestible residues consisting largely of inorganic fragments such as broken setae and occasional sand grains from the gut of the prey are ejected from the anus.

The food reserve

The principal reserve in both species consists of fat which is stored as droplets of up to 2.5 μ diameter in the gastrodermis and parenchyma. In mature females the ova also contain large amounts, in smaller droplets 0.2–0.5 μ in diameter.

Only small amounts of glycogen occur, in well fed individuals, as tiny granules scattered throughout the gastrodermis, parenchyma and musculature.

Other sites of enzymic activity

In *L. ruber* strong non-specific esterase activity, demonstrable by the Gomori α-naphthyl acetate method, occurs in a narrow distal band in the epidermis (Fig. 8) and in the cephalic furrows, foregut epithelium and the endothelium of the blood vascular system and protonephridial excretory system. Alkaline phosphatase and lipase were also found in the walls of the protonephridia, and acid phosphatase in small amounts in some of the epidermal gland cells. Exopeptidases occur in considerable amounts in the endothelial and gelatinous layers of the blood vascular system (Gibson and Jennings, 1967).

In *L. sanguineus,* exopeptidases are found at the sites showing non-specific esterase activity in *L. ruber,* namely the epidermis, cephalic furrows, foregut epithelium, and protonephridia. They occur also in the blood vascular system and in the distal regions of the interstitial cells of the proboscis epithelium. Alkaline phosphatase occurs in the protonephridia and, to a lesser extent, in the gonad endothelia and the ventral wall of the rhynchocoel. Acid phosphatase is found in the epidermal acidophilic glands and also in the material secreted by these glands on to the body surface.

The occurrence of these enzymes, at all the sites named in both species, is quite independent of the nutritive state of the animal.

ENOPLA

Order: HOPLONEMERTINI

Amphiporus lactifloreus

Structure of the gut and proboscis

A. lactifloreus, in common with many other hoplonemerteans, lacks separate external openings to the buccal cavity and rhynchocoel. A small oval aperture on the anterior tip of the body, the rhynchodaeal pore, opens into the rhynchodaeum from which the esophagus leads ventrally and the rhynchocoel dorsally (Fig. 11).

The rhynchodaeum epithelium consists of ciliated cuboidal cells 6–8 μ tall and lies over parenchymatous tissue containing oblique muscle fibers. It lacks gland cells, but mucus-producing cells occur in the underlying parenchyma and discharge through the epithelium around the rhynchodaeum.

The gut is divisible histologically into four parts, the esophagus, stomach, pyloric tube and intestine (Fig. 11). The intestine extends anteriorly beneath the pyloric tube as a blind cecum, and both intestine and cecum bear shallow, paired lateral multilobed diverticula.

The esophagus is a simple tube, 40–50 μ in diameter and about 1 mm long in an adult *A. lactifloreus.* It is lined by ciliated columnar cells 10–12 μ tall and 6–8 μ wide and lacks any glandular components. Posteriorly, the esophagus thickens and opens into the stomach, which has a thick, much folded and highly glandular wall (Fig. 9). This consists of densely ciliated acidophilic columnar cells, 50–70 μ by 5–8 μ, and large numbers of gland cells, 40–50 μ by 12–15 μ, which are loaded with finely granular intensely basophilic secretion. A proportion of the columnar cells appear to be secretory when fully developed, since acidophilic globules are often present in the distal regions and similar globules occur in the stomach lumen. The gland cells discharge either directly into the lumen or proximally between the columnar cells. In the latter case tracts of secretion extend from the gland cells up between the columnar cells and into the lumen.

Posteriorly the stomach wall becomes narrower, less folded and the proportion of gland cells diminishes as the stomach becomes continuous with the pyloric tube. This is non-glandular, lined by ciliated columnar cells, 25–35 μ by 3–5 μ, and somewhat folded in disposition. The tube narrows at its junction with the intestine.

The intestinal wall, or gastrodermis, is similar in structure to that of the other nemerteans studied, in that it consists of acidophilic gland cells packed with proteinaceous spheres and interspersed between ciliated columnar cells. The gland cells are most numerous in the anterior intestine, occurring in the ratio of one to every five columnar cells, but posteriorly the ratio drops to one in fifty.

Physiologically, however, both cell types differ markedly from their counterparts in *Cephalothrix* and *Lineus.* The contents of the gland cells show no reaction whatsoever to either the Hess and Pearse method for endopeptidase or the methods for non-specific esterase. They are discharged during the extracellular phase of digestion in the usual way, and are presumed to be enzymic, but their precise nature remains unknown. The columnar cells differ in that they contain up to 17–18 spherical acidophilic inclusions of variable diameter and these do show intense

positive reactions for endopeptidase. The inclusions are not food vacuoles, as they appear in the basal regions of the cells in the absence of food (Fig. 12), increase in amount with time and are discharged distally as food enters the lumen (Fig. 13).

The proboscis in *A. lactifloreus* is armed with stylets, relatively long and lies coiled in the rhynchocoel for nearly the full length of the body. The rhyncocoel is lined by a thin endothelium, which overlies a muscular layer consisting of inner longitudinal and outer circular fibers.

The proboscis is differentiated into three regions, an anterior basophilic thick-walled tube, a short central bulbous portion housing the stylet apparatus, and a posterior acidophilic thin-walled region. The anterior tube, when retracted, consists of an endothelial layer enclosing four layers of muscle fibers which are, respectively, longitudinal, circular, longitudinal and circular in disposition. The inner epithelium, which forms the outer surface of the everted proboscis, is composed of columnar and gland cells and is thrown up into regularly arranged papillae. The papillae presumably increase the grip of the proboscis on the prey, aided perhaps by the secretions of the gland cells. These, however, are non-mucoid in staining reaction.

The median bulbous portion of the proboscis is in two parts (Fig. 11). The anterior region contains a central needle-shaped stylet, 140–145 μ long and 25–30 μ in diameter at its proximal end, which is borne on a sub-cylindrical stylet base 135–145 μ by 60 μ. The stylet is flanked by paired accessory pouches, each of which may contain up to six accessory stylets in various stages of formation. When complete each accessory stylet has the structure and dimensions of the central stylet. Lateral acidophilic gland cells discharge their secretions at the bases of the stylets and appear to be responsible for the formation of these structures.

The posterior part of the median bulbous portion of the proboscis is composed entirely of muscle fibers arranged around a narrow central lumen (Fig. 14). This opens anteriorly at the base of the central stylet and posteriorly is continuous with the cavity of the third, posterior region of the proboscis. This region is highly glandular, the wall containing both acidophilic and basophilic gland cells, some of which show a reaction for non-specific esterase, and the circular and longitudinal muscle layers are much reduced in thickness. The lumen contains a granular secretion derived from both types of gland cell, when the proboscis is in the retracted position, and it is believed that the secretion is ejected forwards into the wound caused by the central stylet when the proboscis is everted during the feeding process.

The food and feeding mechanism

During extensive laboratory tests the only food accepted by *A. lactifloreus* was the amphipod crustacean *Gammarus locusta*. All other amphipods, isopods and decapods offered failed to elicit a feeding response when presented alive, injured or dead, and the same result was obtained with a variety of oligochaetes, polychaetes and molluscs. No evidence was found to suggest detection of food from a distance and the nemertean appears to rely solely upon chance encounter with living *Gammarus* during random wanderings. When this occurs the proboscis is everted and coils tightly around the crustacean. The stylets penetrate the prey's cuticle and the posterior proboscis secretions pass through the wound into the body cavity.

During capture the *Gammarus* struggles violently but within forty seconds of proboscis eversion it becomes quiescent and the proboscis releases its grip and withdraws into the rhynchocoel. The proboscis is not used to pull the prey towards the head, as in the other species studied, and as it releases its grip so the nemertean moves forward until the head is in contact with the crustacean. The head and anterior region then make exploratory movements over and around the prey, apparently in a search for some weak spot in the integument such as occurs beneath the pleura and coxal plates. The head is then inserted into the prey and the stomach is everted through the rhynchodaeum (Figs. 15 and 16). If the head fails to gain entry the anterior portion of the proboscis is everted once more and applied to the *Gammarus*. It is held in one position for 1–2 minutes, with the end formed into a cup or sucker-like structure, and then withdrawn. The nemertean again attempts to insert its head, usually successfully, but if this is still not possible the procedure with the proboscis is repeated until the integument is breached. It is not known precisely how the proboscis achieves penetration, but it may be by mechanical action supplemented perhaps by some histolytic action of the secretions produced by the gland cells of the anterior proboscis.

The folding of the stomach and pyloric tube, in the resting condition, allows the stomach to be protruded well forwards through the rhynchodaeum and, with the nemertean's head actually within the prey, it is applied to the various organs and tissues of the body cavity. These become disorganized and partially broken down, suggesting the release of histolytic secretions from the glands of the stomach wall although these do not react to any of the histochemical methods employed for enzyme visualization. Portions of the *Gammarus* exoskeleton adjacent to the protruded stomach, however, develop a pink to red coloration, similar to that obtained when they are treated *in vitro* with 0.1 N hydrochloric, sulfuric or acetic acids. Thus it is concluded that a proportion, at least, of the stomach secretions are strongly acidic in nature, a conclusion supported by the intense basophilia shown by most of the stomach glands.

Material from the *Gammarus* appears in the intestine and cecum within three minutes of insertion of the nemertean's head and protrusion of the stomach. Ingestion results from strong, regular contractions of the circular muscles of the general body musculature (Fig. 15). Contractions commence in the region just posterior to the pyloric tube and pass down the body at the rate of 8–12 per minute. Ingestion occupies 30–40 minutes, with the head and stomach moving about within the *Gammarus,* and when completed usually little remains of the prey other than the empty exoskeleton. During the entire process the nemertean retains a firm hold on the substratum by pressing down the posterior third of the body and secreting considerable quantities of sticky mucus from the ventral surface of that region.

Dead specimens of *Gammarus* are ingested in the same manner as living ones, except that the initial proboscis eversion does not take place. Secondary eversion to penetrate the integument may still occur, however, if necessary.

The site and sequence of digestion

Material entering the intestine is at an acidic pH, and generally much disorganized, as a result of the secretions poured on to it from the stomach wall

during ingestion. Carbonic anhydrase activity could not be demonstrated in the stomach or intestine, though, so acid formation in *A. lactiflorens* must involve some process distinct from that found in the other species studied.

The acidophilic gland cells of the gastrodermis discharge as food enters the intestine and cecum but, as noted earlier, it proved impossible to identify the enzymic component secreted. The columnar cells also discharge the spherical inclusions which they accumulate between meals (Fig. 13) and these show a strong reaction for endopeptidase both within the cells and after entry into the gut lumen. This reaction is taken up by the ingested food and as the number of spheres in the columnar cells decreases, there is a corresponding increase in the amount of enzyme activity demonstrable extracellularly.

Five to six hours after ingestion food vacuoles form in the columnar cells and contain material showing endopeptidase activity comparable in intensity to that seen in the gut lumen. The number of vacuoles increases with time, until all material from the lumen has been phagocytosed, but there is no increase in the intensity of the vacuolar endopeptidase activity, so that it can be concluded that there is no intracellular secretion of this enzyme into the vacuoles. Occasional spheres of endopeptidase remain within the columnar cells but these are clearly differentiated from the vacuoles.

Strong acid phosphatase activity occurs in and around the food vacuoles during this stage of intracellular digestion, which lasts for about thirty-six hours. The endopeptidase and acid phosphatase activity then declines and is replaced by exopeptidases and alkaline phosphatase at the same sites. These enzymes persist in the columnar cells until digestion is completed, when they disappear and cannot be demonstrated subsequently until the equivalent stage in the digestion of the next meal. This is the usual pattern for exopeptidase activity, as seen in the other nemertean species studied, but the absence of alkaline phosphatase from the gastrodermis at all times other than the terminal phase of digestion is quite exceptional.

Carbohydrases and lipases could not be demonstrated within the vacuoles, but their presence is inferred from the disappearance of carbohydrate and fatty components of the meal from the vacuoles.

The food reserves

As in the other species examined, fat forms an important food reserve in *A. lactiflorens*. It occurs principally in the columnar cells of the gastrodermis where very large amounts are stored in droplets of up to 4 μ diameter, although occasional globules of 10 μ diameter were found. The only other site of fat deposition is the ovarian endothelium and the mature ova, and it is absent from the parenchyma which is normally a lipid storage region in the nemerteans.

Extensive deposits of glycogen occur in the distal region of the gastrodermis, and lesser amounts in the parenchyma, musculature and gonads.

Other sites of enzymic activity

A narrow band of non-specific esterase activity 2–2.5 μ deep occurs distally in the epidermis and esophagus, and the epidermal mucus glands occasionally show acid phosphatase.

The other enzymes demonstrated in *A. lactifloreus* were exopeptidases associated with the blood vascular system, at the same sites as in *Cephalothrix* and *Lineus*.

Tetrastemma melanocephalum

The structure of the gut and proboscis

T. melanocephalum closely resembles *Amphiporus lactifloreus* in the histological structure of the gut and proboscis. Thus both organs open to the exterior via a common rhynchodaeum, the gut is divided into esophagus, stomach, pyloric tube and intestine, and the proboscis shows the same three regions and stylet apparatus as in *A. lactifloreus*. Such differences as do occur between the two species are only slight, and include a lesser degree of folding in the walls of the stomach and pyloric tube, the absence of paired diverticula from the intestinal ceca and a reduction in the number of acidophilic gland cells present in the gastrodermis. The contents of the gland cells, as in *A. lactifloreus*, show no reaction for either endopeptidase or non-specific esterase and their nature remains unknown.

The columnar cells of the gastrodermis contain varying amounts of acidophilic spheres, which show positive reactions for endopeptidase, and these appear to form in the basal portions of the cells and subsequently migrate distally to accumulate until food enters the intestine. The columnar cells also show food vacuoles, with contents undergoing intracellular digestion, and other vacuoles containing numbers of tiny black granules which are presumably indigestible residues from previous meals.

The food, feeding mechanism and site of digestion

T. melanocephalum proved to be a relatively rare species and only three individuals were available for the whole of this investigation. These three failed to feed upon any of the annelids, crustaceans or molluscs presented to them, and also refused dead animals and organic materials such as clotted blood and liver fragments. The gastrodermal columnar cells, however, consistently showed the aggregations of small black granules already mentioned and interpreted as representing residues of earlier meals. The granules were very similar in size and general appearance to the chloragogenous granules present in oligochaetes, and in the absence of further evidence it is suggested that annelids of this type form the principal component of the diet. It may well be that *T. melanocephalum* has a very rigid dietary preference, and that scarcity of the food organism, therefore, may be the factor controlling the occurrence of the species in any one habitat or at any given season.

The similarities in *T. melanocephalum* and *A. lactifloreus* as regards proboscis structure, stylet apparatus and gut structure all suggest that the feeding process is very similar in the two species. Certainly the folding of the walls in the stomach and pyloric tube in *T. melanocephalum* would appear to allow protrusion of the stomach through the rhynchodaeum, in the manner observed in *A. lactifloreus*.

Since specimens could not be induced to feed it was not possible to study in detail the site and sequence of digestion. However, as in the case of the feeding mechanism, sufficient evidence was available from the gut structure and the enzymes

demonstrable to suggest that digestion follows much the same path as in *A. lactifloreus*. Apart from the acidophilic endopeptidase spheres found in the columnar cells, which are believed to be responsible for extracellular proteolysis, the only other enzymes demonstrable in the gastrodermis were acid and alkaline phosphatase, associated with proximal vacuoles in the columnar cells. No exopeptidase activity was found, though, and there was no distal concentration of alkaline phosphatase in any part of the gut.

The food reserves and other sites of enzymic activity

No observations were made on the nature of the food reserves. Non-specific esterase activity was found distally in the epidermis, and exopeptidases were consistently present in the endothelium, gelatinous layer and plasma of the blood vascular system.

Prostoma rubrum

Structure of the gut and proboscis

The gut in *P. rubrum* is simpler than in the other two hoplonemerteans examined, in that it lacks both a pyloric tube and an intestinal cecum.

The terminal rhynchodaeal pore opens into the rhynchodaeum which is continuous anteriorly and dorsally with the rhynchocoel, and posteriorly with the esophagus. The latter is about 0.1 mm in length, slightly folded, and lined by cuboidal ciliated cells 6–8 μ tall. It opens directly, without any terminal constriction, into the stomach which is a bulbous, thick-walled organ some 200 μ long. The stomach wall is thicker dorsally, deeply folded and composed of three cell types. The commonest is columnar, 25–30 μ by 3–4 μ, with basophilic cytoplasm and densely ciliated distally. Pyriform gland cells, 10–15 μ by 4–5 μ and filled with spheres 0.5 μ or less in diameter, occur between the columnar cells and tracts of secreted spheres extend from them between the columnar cells into the gut lumen. The basophilic glands are PAS positive and the acidophilic ones negative, but the latter show an intense positive reaction for carbonic anhydrase (Fig. 17), indicating that they are concerned in production of acid. Both types are negative to Alcian blue.

Posteriorly the stomach wall becomes somewhat reduced in thickness and the gland cell content diminishes, but there is no clear demarcation into a pyloric tube as, for example, in *A. lactifloreus*. A slight constriction marks the junction with the intestine.

The intestine bears shallow, paired diverticula laterally over most of its length, and its wall is virtually identical with that of the other hoplonemertean species. The columnar cells contain acidophilic endopeptidase spheres, apparently secreted proximally between meals and discharged distally on the entry of food into the intestine, and food vacuoles whose appearance and contents depend upon the time elapsed since the previous meal. The gland cells, occurring in the ratio of 1 : 30 with the columnar cells, are acidophilic and of the usual form and, as in *A. lactifloreus* and *T. melanocephalum*, show no reaction for either endopeptidase or non-specific esterase.

The proboscis has the characteristic hoplonemertean form, being divided into an anterior basophilic region, a median bulbous portion bearing a central stylet and lateral accessory stylets, and a posterior acidophilic region. Histologically the proboscis is very similar to that of *A. lactifloreus,* but two important physiological differences occur. The acidophilic glands of the epithelium covering the anterior proboscis show strong carbonic anhydrase activity (Fig. 17), and a number of the acidophils of the posterior region give a reaction for endopeptidase. The secretion present in the posterior proboscis lumen, in the retracted condition, shows a similar endopeptidase reaction.

The food and feeding mechanism

P. rubrum fed in the laboratory on small living oligochaetes, and, on one occasion, a *Chironomus* larva. Small specimens of *Tubifex* were captured and ingested if the nemerteans were starved for seven days, but other oligochaetes such as *Aeolosoma* and *Stylaria* were taken much more readily. These species were abundant amongst the roots of floating water plants in the aquaria used for maintaining *P. rubrum* and the nemertean itself favored this type of habitat, rather than bottom debris or the leaves of bottom rooted plants. Thus the food in nature may well consist of oligochaetes of the *Aeolosoma* type, which occur in the selected microhabitat rather than those of the *Tubifex* type which are bottom dwellers in silt or mud.

The proboscis is used to capture the prey, being everted after a chance encounter with living oligochaetes. No evidence of either chemical or visual detection of food was found. The everted proboscis wraps tightly around the prey, which ceases movement almost instantaneously. Penetration of the integument by the stylet was not observed, but this and the injection of some paralyzing secretion can be safely inferred from the abrupt death of the prey once the proboscis has made contact. The secretions injected are presumed to be those of the posterior proboscis, which show an endopeptidase reaction, supplemented perhaps by acidic secretions from the anterior portion and originating in the glands rich in carbonic anhydrase.

The proboscis draws the inert prey back through the rhynchodaeal pore into the rhynchodaeum, where the food may be held for a short time while the proboscis is retracted fully into the rhynchocoel, and then passed rapidly through the stomach and into the intestine.

No evidence of stomach eversion was seen and the entire feeding mechanism resembled that seen in the anoplan species (*C. bioculata, C. lincaris, L. sanguineus* and *L. ruber*), rather than that of *A. lactifloreus,* the only other enoplan species seen to feed in the laboratory. Dead oligochaetes, and living or dead crustaceans such as *Gammarus* and *Asellus,* did not evoke proboscis eversion and were not ingested.

The site and sequence of digestion

Since only a small number of specimens were available it proved impossible to study every stage of digestion. Sufficient information was acquired, however, to show that digestion follows the same course as in *A. lactifloreus* with an extra-

cellular proteolytic phase, effected by endopeptidase secreted by the columnar cells, being followed by phagocytosis and completion of digestion intracellularly. The acidophilic gland cells of the gastrodermis discharge during the extracellular phase, but it proved impossible to identify their secretions or determine the part played by these.

The presence of carbonic anhydrase in some of the stomach glands indicates that their secretions are acidic in nature. Since the prey is killed before ingestion the secretions cannot have entirely the same function as those produced in the foregut of the anoplan species and they are not used in any form of extra-corporeal digestion as in *A. lactiflorcus,* but they probably serve to denature the protein component of the food and to provide an acidic medium for the initial, extracellular proteolysis.

The food reserves

Fat occurs in the columnar cells of the gastrodermis, in droplets 3–3.5 μ in diameter and, to a lesser extent, in the gonads. Glycogen occurs at the same sites, with the larger deposits in this instance in the gonads.

Other sites of enzymic activity

Alkaline phosphatase occurs in the walls of the excretory ducts and acid phosphatase in a small proportion of the epidermal mucus glands.

The only other sites showing enzymic activities were the gelatinous and endothelial layers of the blood vascular system, where exopeptidases were consistently present as in all the other species of nemerteans examined.

Discussion

Of the seven species of rhynchocoelans investigated six are seen to be carnivorous and it is likely that the seventh, *Tetrastemma melanocephalum,* will also be found to take animal food, judging from the structure of the proboscis and alimentary canal and the occurrence of endopeptidase in the gastrodermis. The proboscis is used to capture active living prey, either with or without the supplementary use of stylets and an accompanying injection of paralyzing secretions, but inert or dead food if eaten at all is ingested directly and does not stimulate proboscis eversion. The digestive physiology, with strong emphasis on proteases and, in the majority of species, production of acid anteriorly in the gut, is clearly adapted to a predominantly animal diet. Similar findings on one or more of these aspects of feeding and digestion have been reported in the Anopla for various species of *Lineus* (McIntosh, 1873–74; Verrill, 1888–1892; Riches, 1893; Wilson, 1900; Piéron, 1914; Coe, 1943; Beklemishev, 1955; Tucker, 1959), *Carinoma, Parapolia, Tubulanus,* and *Zygeupolia* (Coe, 1943) and *Hubrechtella* (Hylbom, 1957). In the Enopla, too, similar findings have been reported for the hoplonemertine genera *Ototyphlonemertes* (Corrêa, 1948), *Geonemertes* (Hickman, 1963) and *Paranemertes* (Coe, 1901, 1943; Roe, 1967).

Thus it would appear that the situation found in the species investigated in the present study represents a pattern of nutrition characteristic of the Palaeonemertini and Heteronemertini in the Anopla, and of the Hoplonemertini in the Enopla.

The Enopla, however, includes a second order, the Bdellonemertini, which contains the single genus *Malacobdella* and this has been shown to be microphagous with a very high proportion of plant material in the diet (Gibson and Jennings, 1969). The basic feeding mechanism is completely different from that of other rhynchocoelans and has involved elaboration of the foregut into a pharynx which is used as a filtration mechanism. The digestive physiology is correspondingly modified and considerable emphasis is placed upon carbohydrases instead of proteases.

Thus in the Rhynchocoela there is a fairly intimate relationship between the diet, on the one hand, and the feeding mechanism, structure of the gut and digestive physiology, on the other; a situation very similar to that seen in the related Turbellaria (Jennings, 1957, 1968), monogenetic trematodes (Halton and Jennings, 1965) and digenetic trematodes (Halton, 1967).

Within the general pattern of nutrition in the carnivorous rhynchocoelans interspecific differences lie principally in the feeding mechanisms and one aspect of the digestive physiology, namely the site of production of the extracellularly acting endopeptidase.

With regard to feeding mechanisms, the main variation from the normal type is seen in *Amphiporus lactifloreus*. Here the head actually penetrates the captured *Gammarus* and the stomach, which is not present in the anoplan species, is everted to achieve first disintegration and then ingestion of the body contents. This type of feeding is well suited to crustacean prey which are enclosed in a tough protective exoskeleton and allows the rhynchocoelan to feed on *Gammarus* manifestly too large to be swallowed intact. Ingestion of large prey is within the capabilities of most of the species studied, but it is significant that in every case observed the ingested animal was either an oligochaete or polychaete annelid, with a relatively soft integument. *Lineus ruber* has been observed, on occasion, to ingest crustaceans intact but these were always very small relative to the rhynchocoelan and did not distend the body as did, for example, a large oligochaete. Presumably large crustaceans would prove resistant to digestion and *L. ruber* never attacked these. *A. lactifloreus*, however, kills *Gammarus* much larger in diameter than itself and is able to ingest the soft internal parts by virtue of its specialized feeding mechanism. In this respect, the stomach in *A. lactifloreus* is directly comparable with the protrusible plicate pharynx of triclad and polyclad Turbellaria, which is thrust into animals such as crustaceans, annelids, insect larvae and even colonial ascidians and used as a suction tube to withdraw body contents (Jennings, 1957, 1959).

Prostoma rubrum possesses the same type of alimentary canal as *A. lactifloreus*, with a well developed stomach, but does not prey on crustaceans. Oligochaetes are the chosen food organisms and the most effective way of dealing with these is apparently to ingest them intact. Eversion of the stomach does not occur and the entire feeding process resembles that seen in the Anopla.

Other differences in the feeding mechanisms of the species studied are relatively minor and are seen in the presence or absence of proboscis stylets. The Anopla lack stylets but in the two species of *Cephalothrix* the proboscis epithelium contains rhabdite-like barbs which apparently serve the same function as the more elaborate and larger stylets characteristic of the Enopla. Other, fluid, proboscis secretions are believed to be toxic, like those of the posterior proboscis in the Enopla, and the prey is killed during capture. In *Lineus sanguineus* and *L. ruber* barbs are present,

but despite this the prey is ingested alive. Death occurs in the foregut, though, as the prey passes through and is exposed to the acidic secretions produced there.

Variation in the site of production of the extracellularly acting endopeptidase is the principal difference in the digestive physiology of the Anopla and Enopla. The gastrodermal gland cells produce this enzyme in the Anopla, but in the Enopla these glands, although virtually identical in structure and staining properties, show no reaction histochemically for any type of protease. In the Hoplonemertini (*A. lactifloreus, T. melanocephalum* and *P. rubrum*) production of endopeptidase for use in the intestinal lumen has apparently been taken over by the columnar cells but these still retain their phagocytic properties and digestion is completed within them in a sequence identical with that seen in the Anopla. The gland cells discharge their products into the gut lumen, but the part played by these remains unknown. In the other enoplan order, the Bdellonemertini, the diet contains a much larger proportion of carbohydrate and the gland cells are believed to produce amylases (Gibson and Jennings, 1969). Thus physiological modification of the gastrodermal gland cells, from the form seen in the Anopla, would appear to be characteristic of the Enopla, but it is difficult to see the functional significance of this in the Hoplonemertini which are entirely carnivorous.

The gastrodermal columnar cells in both the Anopla and Enopla are uniformly ciliated, but despite this they are also phagocytic. Conclusive proof that the contents of the food vacuoles result from true phagocytosis, rather than from concentration of absorbed soluble materials, was given by Jennings (1960) and Gibson and Jennings (1969), who showed that starch grains appear in the food vacuoles unaltered in both staining properties and optical activity.

This occurrence of phagocytosis in ciliated cells is of relatively rare occurrence in the animal kingdom and its significance in the Rhynchocoela remains uncertain. A study of the fine structure of the columnar cell has been undertaken to investigate this phenomenon and will form the basis of a subsequent account.

The food reserves in both Anopla and Enopla consist mainly of fat, a situation characteristic of most free-living animals, but no special modifications for storage or subsequent utilization occur.

Of the enzymes found at sites other than in the alimentary system the only types consistently present in all species are exopeptidases in the blood vascular system. A possible interpretation of this phenomenon, in terms of a peptide circulation analogous to the exocrinic-enteric circulation of amino acids described for the vertebrate intestine by Read (1950) has been fully discussed elsewhere (Gibson and Jennings, 1967).

Fisher and Cramer (1967) report that *Lineus ruber* can absorb amino acids and glucose across the epidermis, which possesses microvilli-like structures interspersed between the cilia. In view of these findings, it is possible that the nonspecific esterase found in the epidermis of *Cephalothrix bioculata, C. linearis, Lineus ruber, Amphiporus lactifloreus* and *Tetrastemma melanocephalum,* and the exopeptidase activity at the same site in *L. sanguineus,* may be concerned with extracorporeal digestion of simple proteins or polypeptides. The products of this digestion, if absorbed across the epidermis, would supplement the normal diet and their transfer about the body and subsequent metabolism may well be linked with the exopeptidase-peptide circulation complex of the blood vascular system.

Our grateful thanks are due to Dr. John J. Poluhowich for collecting and supplying living specimens of *Prostoma rubrum*.

Summary

1. A comparative study has been made of the food, feeding mechanisms, gut structure, digestive physiology and food reserves of representative species from three of the four orders of Rhynchocoela (Anopla: Palaeonemertini and Heteronemertini; Enopla: Hoplonemertini).

2. Polychaete or oligochaete annelids form the staple diet in the Anopla and one species of Enopla. *Amphiporus lactifloreus* (Enopla) is exceptional in that it feeds exclusively on the amphipod crustacean *Gammarus locusta*.

3. Living prey are captured by means of the proboscis, but inert or dead foods, when taken, are ingested directly without proboscis eversion.

4. In the Hoplonemertini the proboscis is armed with stylets and toxic secretions which kill the prey before ingestion, and a forerunner of this system is seen in the Palaeonemertini where minute epithelial barbs perforate the prey's integument to allow entry of paralyzing substances. In the Heteronemertini, though, the prey is swallowed alive and killed by acid secretions in the foregut.

5. In the Hoplonemertini the gut has an additional portion, the stomach, and in *Amphiporus lactifloreus* this is everted within the prey which is then ingested in fragments.

6. The basic digestive physiology is similar in both Anopla and Enopla, with extracellular acidic proteolysis being followed by phagocytosis and completion of digestion intracellularly by proteases, carbohydrases and lipases acting in concert. Intracellular digestion is in two phases, firstly acidic and then alkaline, and acid and alkaline phosphatases are associated with the appropriate phase.

7. The endopeptidase responsible for extracellular digestion is produced in the Anopla by gastrodermal gland cells but in the Enopla this function has been taken over by the columnar cells of the gastrodermis. Functional gland cells still occur in the Enopla but their role has not been determined.

8. The food reserves consist mainly of fat, stored in the gastrodermis in all species and, occasionally, in the parenchyma.

9. These findings are discussed in relation to previous work on nutrition in the remaining order of the Rhynchocoela (Enopla: Bdellonemertini).

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